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EDITORIAL

BOVINE MASTITIS

THE incidence and types of bovine mastitis vary throughout the world. It is acknowledged, however, that the economic loss from the disease as a whole is large and that its control or eradication is followed by increases in milk production. Research work has been intensified in different countries with the result that measures are now in operation whereby the incidence of at least some forms can be markedly reduced. The knowledge that the sterilization of the udder is followed largely by much reduction of the disease has led to the adoption of techniques whereby suitable agents are introduced directly into the udder through the teat canal. The agent of choice at the present moment for action against streptococci is penicillin: undoubtedly new antibiotics and chemotherapeutical substances will be found which will be effective against a wider variety of the micro-organisms associated with mastitis. Recent publications indicate that even now antibiotics other than penicillin are giving results of high order. This method of controlling and eradicating mastitis is an example of how modern methods can be applied to diseases of livestock and how veterinarians make use of the discoveries in the scientific world.

Any new information on the causes and treatment for mastitis is valuable: especially is this so in connection with the forms which as yet do not respond well to any of the treatments which have been used. The method of attack through the teat canal may not apply equally well to all the micro-organisms found in the udder of cows, and it may well be that we have to explore other techniques for the control of some forms of the disease.

Although we are now in a much better position to deal with mastitis than we have ever been in the past and can be of valuable assistance to farmers and others by the application of the present-day techniques, it is still necessary to have a better understanding of the fundamental or basic features of the disease. All cows in a herd do not suffer from mastitis: in the udders of some the recognised micro-organisms may be present for long periods and yet clinical mastitis does not develop. Some cows, although exposed equally to the infection

which gives rise to severe disease in others, do not become infected over long periods of exposure. We have still to become acquainted with the reasons for the variability of the incidence of the disease in the different herds. Having now reached the stage when some of the forms of mastitis can be controlled or eliminated, the research worker is in a position to devote more of his time to some of the more fundamental work on the subject. Herd management will have to be looked into and due attention will have to be given to the many small differences which are found in it throughout the country. By making the necessary comparisons of herds in which the occurrence of mastitis in a clinical form is prevalent as well as the extent to which udder infections exist with those in which they are rare, will give a lead to further inquiry into their association with management and other causes.

It is also necessary to study more fully the udder tissue itself and the course of the infections in it. More histological examinations will have to be made at different stages of udder development and of lactation. The effects of the various hormones on the susceptibility of the udder tissue to infection deserves consideration. There is an extensive and interesting field of work open for the research worker. It may be that, following investigations on such and other lines it will be possible so to alter husbandry and dairy methods that mastitis can be controlled without the application of the types of treatment now being practised. These are ideas for the future: in the meantime, it is hoped that the known methods will be used on an ever-increasing scale for the benefit of the dairy farmer and the milk supply.

GENERAL ARTICLES

BOVINE MASTITIS
Associated with Beta-Hœmolytic Group C Streptococci

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THE occurrence of bovine mastitis associated with group C streptococci (*Str. pyogenes* [animal] or *Str. zooepidemicus*) would seem to be rare, since there are few records of its appearance. The available information shows that disease of the udder set up by these organisms is usually sporadic and produces marked clinical symptoms in affected cows, but there appear to have been few recorded instances of this type of mastitis having affected more than a very small number of animals in one herd.

Plastridge and Hartsell (1937) tested serologically five strains of strongly hæmolytic streptococci obtained from milk, and found that three fell within group C. Out of a total of 68 samples of raw milk obtained from various centres, Sherman and Niven (1938) found that 11 contained group C ("animal *pyogenes*") streptococci, and Gunnison and others (1940) recorded the isolation of "animal *pyogenes*" streptococci from one out of a total of over 400 samples of milk. Stableforth (1939) found four cases of mastitis associated with these organisms which had arisen in one herd, and Little and Plastridge (1946) refer to a herd in which group C streptococci spread to 10 out of 15 cows.

There would seem to be a close relationship between *Str. pyogenes* (animal), the "human" group C streptococci and *Str. equi* (the causal organism of strangles), all of which fall within group C. The cultural habits of these three members of the group are similar and their differentiation is based mainly upon their action in trehalose and sorbitol. In general, the human strains of group C hæmolytic streptococci are distinguished by fermenting trehalose but not sorbitol, whilst the majority of those from animal sources ferment sorbitol but not trehalose, with the exception of *Str. equi*, which ferments neither (Wilson and Miles, 1946).

In the following report an account is given of two outbreaks of mastitis in widely separated herds, in each of which a comparatively high percentage of the cows were excreting hæmolytic group C streptococci in their milk and in which subacute or chronic mastitis of a severe kind was present.

Herd I

This herd was situated in Leicestershire. History showed that clinical mastitis had been a source of trouble for some years.

At the time of the investigation the herd contained 46 cows in milk, of which many were showing clinical evidence of mastitis, with induration of the quarters and abnormal secretions. A number of cows had ceased prematurely to lactate, whilst others had yielded milk only for a short time. In spite of apparently adequate treatment with penicillin of clinical cases by the veterinary surgeon, it was evident that disease was increasing throughout the herd.

Methods

Bulk milk samples were taken from the 46 cows into sterile screw-capped bottles and were conveyed direct to the laboratory. Upon arrival, 0.1 c.c. of whole milk from each sample was spread upon the surface of a bovine blood agar plate. Petri plates were then incubated for 48 hours at 37° C., after which colonies were examined and their number estimated. The remainder of each milk sample was incubated for 18 hours at 37° C., following which one loopful of milk was streaked on to one half of an Edwards plate (crystal violet, æsculin, blood agar, Edwards, 1933) and the results were recorded after 24 hours' incubation.

1. Bacteriological Findings

Of the 46 cows in the herd, 32 were found to be carrying hæmolytic streptococci, by the direct culture method, and all of these showed infection amounting to approximately 1,000 or more colonies of streptococci on blood agar after 48 hours' incubation. Milk samples from nine cows showed slight infection by the growth of hæmolytic streptococci on Edwards' medium after incubation of the sample.

2. Group C Streptococci

It was noticed upon further examination of culture plates that, whilst streptococci from all positive samples showed beta-hæmolysis, those from certain cows exhibited wider zones of clearance and resembled group C streptococci observed from a previous herd outbreak of mastitis. Precipitin tests, using acidified extracts of the organisms against group specific streptococcal antisera, showed that nine of the heavily-infected cows were carrying wide-zoned beta-hæmolytic group C streptococci. The remainder of the infected cows were excreting group B hæmolytic streptococci (*Str. agalactiæ*). Since it was not possible at that time to check further the identity of the group C streptococci, cultures were sent to Mr. J. E. Lancaster of the Veterinary Laboratory, Weybridge, who kindly carried out biochemical and serological tests of the strains. Five strains were tested and all yielded the following results:

Lactose	+
Mannite	—
Salicin	+
Raffinose	—
Inulin	—
Saccharose	+
Trehalose	—
Sorbitol	+
Litmus Milk	Acid
Methylene Blue Milk 1/20,000	—
„ „ „ 1/10,000	—

(a) *Hæmolysis*

In this investigation the power of the group C streptococci to bring about

rapid and complete hæmolysis of both blood agar and Edwards' medium provided their most striking characteristic. The beginning of lysis was discernible after only a few hours' incubation, at which time no colonial growth was visible to the naked eye. Following a period of 18 hours' incubation at 37° C., there was a wide zone of complete lysis around somewhat discreet colonies. The diameter of the hæmolysis varied between four and six millimetres and a well-marked zone of partial hæmolysis at the perimeter was a constant cultural feature of the group C streptococci.

(b) *Cultural characteristics*

The group C streptococci showed great rapidity of growth by comparison with other mastitis organisms. Colonies on solid media were visible after 10 hours' incubation and appeared to have reached the maximum of their development by the 48th hour, at which time they presented a somewhat gelatinous appearance with abruptly raised central zones. At this time, growth on Edwards' medium showed no tendency to take up the crystal violet and remained colourless or grey. Growth in 1 per cent glucose broth was rapid and heavily flocculent, whilst nutrient broth proved a less advantageous medium.

The biochemical reactions have already been described and these were checked later by the author, with the same results.

(c) *Morphology*

Microscopic examination of smears from liquid culture media showed chains varying in length from 12 to 20 cocci, with the formation of occasional " palisade " types of cells. Following growth on solid media, chains appeared shorter, with a greater tendency towards the formation of " palisade " cocci.

(d) *Precipitin reactions*

Acid extracts of the organisms reacted strongly with group C streptococcal antiserum, producing a clearly defined zone of precipitation within four minutes. The positive reaction obtained with group II antiserum (*Str. dysgalactiæ*) was less marked. Tests against antisera of other streptococcal groups were negative.

Treatments

In view of the serious and widespread nature of the outbreak, it was decided to commence penicillin treatment with the least delay and at the same time to compare the results between the two infections in the same herd. This was effected by the administration of courses of treatment, each lasting three days, followed by suitable intervals to allow time for the removal of penicillin from the udders before bacteriological tests were made. Three such courses of treatment were given.

Each infected cow received treatment consisting of 50,000 units of penicillin as a solution in 50 c.c. of boiled water into all quarters of the udder once daily for three successive days. Following treatment, a period of three days was allowed to elapse before quarter samples were taken for examination. Following the bacteriological examination after treatment, all cows which

showed infection with streptococci in any quarter were given a further three days' course of treatment similar to the previous one. Three such treatments were administered and the results are shown in Table I.

Results

Bacteriological tests were carried out on uninfected cows at intervals throughout the investigation; a small number of these became infected and were treated, with good results, but in the majority of cases cured udders remained apparently free from organisms throughout the period of treatment. It was noticed that the few cows which became reinfected after treatment produced streptococci of the same zoological group as those with which they had been infected originally, thereby showing, probably, that streptococci were yet present in the udders following treatment, but in numbers insufficient to be shown at the time of sampling, either by direct or by enrichment methods of cultivation.

Although the pre-treatment test showed that a total of 41 cows out of 46 were carrying hæmolytic streptococci in their udders, the number of quarters infected at that time is not known, since only bulk samples from each cow had been taken. In view of the interest attached to the results of this test, it was decided to obtain quarter samples from all cows subject to further tests, and in order to economise as far as possible with labour and materials, the main investigation was confined to the cows originally infected. It was decided to carry out check tests of uninfected cows by bulk udder samples at intervals.

TABLE I

Showing the results of three courses of treatment with penicillin

(HERD I)

Time of Test	GROUP B STREPS. Heavy C	Q	Medium C	Q	Light C	Q	Total C	Q	GROUP C STREPS. Heavy C	Q	Medium C	Q	Light C	Q	Total C	Q
Before treatment ...	21		1		9		31		... 9		1		0		10	
After 1st treatment ...	2	2	1	4	4	5	7	11	... 8	15	1	1	1	3	10	19
After 2nd treatment ...	2	2	0	0	5	8	7	10	... 3	7	0	0	2	5	5	12
After 3rd treatment ...	1	1	0	0	2	2	3	3	... 2	2	0	0	0	0	2	2

NOTE.—C = Cows.
Q = Quarters.

Table I shows the results of treatment upon the 41 cows found originally to be infected with streptococci. Thirty-one of these were infected at pre-treatment test with *Str. agalactiæ* and 10 were excreting group C streptococci. Infections with both groups of organisms were graded as "heavy" or "medium," according to the estimated number of colonies visible following the incubation of direct culture plates for 48 hours at 37° C. Infections were classed as "light" when streptococci grew only on Edwards' medium following incubation of the milk sample. By this method an estimated count of over

1,000 colonies was taken as a heavy infection, and less than this number as a medium infection.

Quarter samples were examined following the first course of treatment, when it was found that, of the cows infected with *Str. agalactiæ*, 24 had responded and that seven were still infected in 11 quarters; whilst, of the cows carrying group C streptococci, no diminution in the total number had taken place, although one animal which had previously been heavily infected showed streptococci on Edwards' medium from three quarters. Following the second course of treatment, the total number of quarters infected with *Str. agalactiæ* had been reduced by one. It was general for cows with light infections to become free after one course of treatment, and as the investigation proceeded it was evident that, although a few were becoming reinfected, the general picture was one of progress.

It was not until two courses of treatment had been given that any considerable lessening in the incidence of group C infection was apparent. At the second post-treatment test the number of heavily infected quarters had been reduced from 15 to 7, and the total number of infected cows from 10 to 5. Following the third course of treatment on cows still infected, only three quarter samples of three cows yielded heavy plate counts, and of these two were infected with group C streptococci and one with *Str. agalactiæ*. The investigation was discontinued at this point and treatment or disposal of cows still infected was left to the veterinary surgeon in charge. A herd test carried out one month later showed six cows heavily reinfected with *Str. agalactiæ* and one with group C streptococci.

Discussion

From results obtained in Herd I it seemed probable that under ordinary field conditions difficulty might have been experienced in carrying out successful treatments of group C infections. In circumstances where clinical alteration of milk forms the basis of diagnosis and treatment is often restricted to not more than three daily infusions of 50,000 units of penicillin, it is questionable whether a satisfactory response would have been shown in this herd.

Resulting from the first course of treatment, the number of heavy and clinically evident infections caused by *Str. agalactiæ* was reduced by 90 per cent, whilst those due to group C streptococci were lowered by 11 per cent (Table I). In general, it took three courses of treatment in cows heavily infected with group C streptococci to reduce the incidence of infection to a level which had been reached in two similar treatments of cows carrying approximately the same degree of infection with *Str. agalactiæ*. Figures given in Table I for light infections are of less significance, since they include cows which showed recurring infection and are of interest only in that they had been reduced at the end of the investigation.

Herd II

This herd is maintained in Lincolnshire, and at the time of the investigation there were 35 cows in milk, of which a large number were showing evidence

of mastitis, with clinical alteration of milk. The veterinary surgeon in charge had been treating clinical cases with penicillin, but little progress had been made.

Quarter milk samples were taken from all cows and were cultured by the method described for Herd I.

Bacteriological Findings

Of the 35 cows in milk, 25 were found to be infected with streptococci in some degree. From these, 18 quarter samples from nine cows showed colonial growth resembling *Str. agalactiæ,* whilst 16 cows exhibited infection in 41 quarters with wide-zoned beta-hæmolytic streptococci, typical of the group C streptococci of Herd I. The identities of both types of organisms were confirmed later by serological and biochemical tests.

Treatments

Following the experience gained with the treatment of Herd I, a further attempt was made to compare the effects of penicillin upon the two infections in the same herd, with three daily infusions of the udder, but with an increase to 100,000 units of penicillin in 50 c.c. of boiled water.

For the purposes of this investigation the results of treatment were assessed on 12 cows which were showing heavy infection in 15 quarters with *Str. agalactiæ* and in 12 quarters with group C streptococci. In this group of animals no infected quarters showed, before treatment, an estimated direct plate count of less than 10,000 colonies on blood agar following incubation for 48 hours at 37° C.

TABLE II

Showing the effects of treatment on 12 heavily infected cows

HERD II

Time of Test	GROUP B STREPS. Heavy C	Heavy Q	Medium C	Medium Q	Light C	Light Q	Total C	Total Q	GROUP C STREPS. Heavy C	Heavy Q	Medium C	Medium Q	Light C	Light Q	Total C	Total Q
Before treatment ...	7	15	—	—	—	—	7	15	... 5	12	—	—	—	—	5	12
After 3 x 100,000 units penicillin	—	2	—	—	—	3	2	5	... —	4	—	1	—	—	2	5

NOTE.—C = Cows.
Q = Quarters.

Results

Table II shows the results of the penicillin treatment upon 12 heavily infected cows possessing approximately equal degrees of infection with group B and with group C streptococci, and providing between them a nearly equal number of quarters infected with each type of streptococcus.

Following treatment, quarter samples were taken after four days from the date of the last treatment for further bacteriological examination. It was found that the number of quarters infected with *Str. agalactiæ* had been reduced by 66 per cent from 15 to 5. Two quarters were showing, at this time, no estimable diminution of infection, whilst three quarters were showing

infection only by enrichment methods of culture. Quarters infected with group C streptococci had been reduced by 58 per cent, from 12 to 5, and four quarters were found still to be heavily infected, whilst one quarter gave a direct plate count of approximately 1,000 colonies.

The rest of the infected cows in the herd, which amounted to two infected with *Str. agalactiæ* and 11 infected with group C streptococci, were treated apart from this investigation.

General Discussion

Extensive outbreaks of bovine mastitis caused by *Str. pyogenes* (animal) group C apparently are rare and reference can be found to only one other, reported from America. Since, however, two such outbreaks were seen in the same region of England within a period of a few weeks, it is possible that this form of the disease may be more widespread than is suspected. In any event, its occurrence is not unknown in this country since, in collaboration with other workers, the author studied a similar outbreak some years previously (unpublished data). However, its incidence cannot be high in the East Midlands, since the bacteriological examination of over 2,000 milk samples from cases of suspected mastitis received at the laboratory have failed to reveal any additional cases of this type of the disease.

In the two outbreaks under review there was a difference between the clinical manifestations of group B and of group C mastitis, and although a differential diagnosis could not have been made, with safety, on such grounds alone, the type of disease caused by group C streptococci appeared to be the more severe. In view of the lack of response which this type of mastitis showed to penicillin treatment when compared with that generated by *Str. agalactiæ*, there would appear to be grounds for the investigation of certain herd outbreaks of the disease which fail to respond to a recognised course of treatment. Whether the results here reported were due to a greater resistance on the part of group C streptococci to penicillin or to differences in the clinical conditions within the udders between the two infections is not known. In neither of the two herds was infection with either organism completely eliminated, but the results showed that, apart from a small percentage of apparently "incurable" cows, *Str. agalactiæ* mastitis responded to penicillin treatment more readily than did that associated with group C streptococci.

Summary

In two widely separated herds heavy udder infections with beta-hæmolytic group C streptococci (*Str. pyogenes* [animal]) caused subacute to chronic mastitis. In both herds mastitis due to *Str. agalactiæ* co-existed. Infections with group C streptococci in the two herds amounted to 25 and 64 per cent of infected cows, respectively.

In the first herd three daily infusions, each of 50,000 units of penicillin in boiled water, reduced infections caused by *Str. agalactiæ* by 80 per cent, but did not alter the total number of cows infected with group C streptococci.

Three such courses of treatment showed a slower response on the part of group C organisms.

In the second herd, in which three daily infusions each of 100,000 units of penicillin were administered to a nearly equal number of quarters infected with group B and with group C streptococci, carrying approximately the same degree of infection, a more even response to treatment was shown.

It is suggested that under ordinary conditions of field diagnosis and treatment, heavy infections of the udder with group C hæmolytic streptococci might fail adequately to respond.

Although small differences were noticeable between the clinical manifestations of the two types of mastitis, it is not known whether the lack of response to treatment shown by group C streptococci was a result of pathological differences in the udders between the two infections or to penicillin resistance on the part of group C streptococci.

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CAUSES OF STERILITY IN BULLS IN SOUTHERN ENGLAND

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(Continued)

(d) *Semen examination.*—Semen was found normal in all cases, except that of AB 7, which was grossly infected with *C. pyogenes* and, to a small extent, staphylococci. Comparatively low motility, 60 x 3, of the first ejaculate of AB 29 was attributed to a long period of abstinence.

(e) *Clinical examination.*—The penile layer of the preputial lining was

apparently normal in AB 7, nodular and congested in AB 19, and very markedly nodular (bold nodules of chronic inflammation) and congested (glans penis extremely reddened) in AB 29.

Since a clinical examination of the prepuce does not ordinarily reveal the depth of the lining to which the inflammatory process has extended, and in some cases fails to show an abnormality of the prepuce altogether, it appears desirable to examine the preputial washings as a routine measure in examining bulls (Haq and Rollinson, 1948).

(4) *Paunchiness*

This was responsible for causing partial or complete sterility in three cases or 9 per cent of the bulls examined. The figure is quite high for a disorder which results merely from injudicious feeding. That an abnormally large and pendulous abdomen can result in *impotentia cœundi* and render a bull completely useless for breeding purposes does not seem to have been realised by many farmers.

(a) *History.*—The outstanding points in history were progressive sluggishness at service, faulty feeding and no exercise. The daily ration comprised hay 35-40 lb. or more, greens and roots 20-25 lb., and concentrates 3-5 lb. Fertility rate was normal, excepting AB 2, which was completely unable to serve.

Abnormally large and pendulous abdomen resulting in *impotentia cœundi* is an extreme instance of injudicious feeding, particularly a large amount of fibrous material, with no provision for exercise.

(b) *Behaviour at service.*—It was "7" in AB 2 and "4"-"5" in the other two cases. The vice of masturbation in AB 2 was the result of idleness and preputial irritation; it disappeared when the latter factors ceased to operate (Rollinson and Haq, 1948).

(c) *Semen examination.*—Semen appeared normal in all respects.

(d) *Clinical examination.*—The abdomen was moderately large in one and very large and pendulous in the other two cases. That of AB 2 was only about 10 in. above the ground plane.

(5) *Faulty erection*

This disorder accounted for sterility in three cases or 9 per cent of the bulls examined.

(a) *History.*—As none of the bulls could reach the cow's vagina, fertility rate cannot be considered. In two cases the condition followed a fall (AB 3 fell into a ditch and AB 41 fell over a barbed wire fence), while in the third case there was nothing to include or exclude the possibility of an accident, the condition being discovered when the bull started his breeding career. Feeding and management of these bulls were not different from those previously described.

In order to ascertain further the link between imperfect erection and mechanical injuries, it is worth while inquiring into the past history of accidents in every case.

(b) *Behaviour at service.*—The bulls mounted the cows with great keenness, but the penis did not erect beyond a length of 3-6 in. In AB 3 it did not come

out of the prepuce at all after the first collection by means of an artificial vagina. Penile rigidity was normal in one and subnormal in the other two cases.

(c) *Semen examination.*—Semen was obtained only from AB 3 and was found normal.

(d) *Clinical examination.*—On walking, all three bulls showed lameness of the right hind leg (on each step the leg was lifted with a jerk combined with extra flexion at the hock, and put down on the ground with hesitation) and stiffness and indifferent action of the lumbo-sacral region. AB 3 also showed sensitiveness of the proximal part of its perineum. In AB 24 the testicles were three-fourths the normal size, while the penis had subnormal rigidity and thickness.

(e) *Autopsy.*— This was performed only on AB 3. The skin over the lumbo-sacral region was thickened and adherent to the underlying parts and showed marked fibrosis. The parts of the semimembranosus muscle, covering the ischiocavernosus muscle, were lacerated and œdematous. A deeper examination of the vertebral and pelvic joints could not be carried out because of the regulations of the Ministry of Food.

(f) *Discussion.*—The imperfect erection with subnormal penile rigidity, followed in all cases by a peculiar lameness of the right hind leg and stiffness of the lumbro-sacral region, most probably resulted from accidental injuries to the lumbro-sacral and perineal regions. A marked fibrosis of the vertebral column and the laceration and œdema of the semimembranosus muscle found on the autopsy of AB 3 gave some evidence of this. AB 23, a case of testicular degeneration, showed also a stiffness of the lumbar region and lameness of the right hind leg as a result of a fall into a river, although his erection appeared normal. Injuries to the lumbro-sacral region can affect erection by interfering with the lumbo-sacral plexus and nervi erigenties transmitting nervous impulses to the genitals. Injuries to the proximal part of the perineal region can damage the ischiocavernosus muscle and other soft structures like the suspensory ligament of the penis and crura, and thereby cause interference with the propulsive mechanism.

The possibility of damage to the retractor muscles of the penis causing imperfect erection (Loje, 1943; Siegel, 1946) was excluded on the strength of the clinical examination of AB 41 under epidural anæsthesia and the post-mortem examination of AB 3. There were no signs of peri-perineal abscess. (Francis, 1947). The possibility of injuries to the ileo-sacral joint resulting in *impotentia cœundi,* especially in young bulls (Forssell, 1935), was excluded in view of a normal service behaviour and faultless jumps in all the three bulls examined. Evidence of neo-pallial injuries, which has been stated to cause impotence and sterility (Beach, 1947), could not be obtained in any of the bulls examined. It was therefore most likely that the imperfect erection of AB 3 and 41 was the result of accidental injuries. As regards AB 24, although there was no history of an accident, evidence to the contrary was also lacking. The small size and soft consistency of his testicles (a progressive decrease noticed by the owner), however, left some doubt as to the possible origin of this condition in the testicles.

In determining the possible effects of injuries to the lumbro-sacral and ileo-sacral joints, it will be worth while investigating which of these two parts is more important. The present investigation shows that the injuries to the lumbro-sacral joint are more important than those to the ileo-sacral joint. Mitchell (1935) has shown that the testicular afferents in man enter the cord at a higher level than is commonly supposed. Similar information in bulls may help to determine the significance of mechanical injuries with greater assurance.

(6) *Unknown disorder*

AB 40 was a case of *impotentia cœundi*. He took no interest in the cow (in heat) and showed no inclination to serve her. His semen, therefore, could not be collected. The clinical examination revealed two points. Firstly, he was in an over-fed beef condition with large fat deposits around the spermatic cord and in the proximal part of the scrotum. Secondly, there was no arrangement for giving him exercise of any kind.

He is still under observation and the cause of his impotence is as yet undetermined. There is, however, a likelihood of the condition being caused by: (1) an excessive infiltration of the body tissues with fat or auto-intoxication leading to inactivity or producing a hypogonadal state (Rice, 1942); or (2) in interference with the functions of the thyroid gland, which in turn has its specific effects on the pituitary and gonadal cells, or general calorigenic effects on all cells including those of the endocrine system (Engle, 1946b).

D. Additional Notes

(1) *Treatment of infertile bulls*

(a) *Testicular degeneration.*—An attempt to treat testicular degeneration was made in six bulls: three with PMS (pregnant mare's serum) and three with ascorbic acid. PMS was selected for the beneficial effects of the follicle stimulating hormone on the seminiferous tubules and of the interstitial cells stimulating hormone on the cells of Leydig producing the male hormone (McKercher, 1942; Turner, 1945). Ascorbic acid was tried on the evidence of its beneficial effects, which hitherto remain unexplained (Phillips, 1940; Klussendorf, 1941). The choice of bulls for a trial of these therapeutic agents was on a random basis, as there is no information to suggest the use of either of them in any particular type of sterility. The planning of a rational treatment was made difficult because of lack of knowledge of the precise etiological factors responsible for testicular degeneration in each case.

(i) *PMS.*—This was tried in AB 6, 8 and 32. Before administering PMS, the genitalia of the bull and its semen were examined to ensure that the condition had not advanced very far and that there was enough healthy testicular tissue to respond to this treatment. This precaution was taken in view of the harmful effects of gonadotrophins noticed in cases of advanced spermatic dysplasia in man (Williams, 1943). The dose varied from 750 to 2,000 units and was given intramuscularly every week for 3-6 weeks.

The administration of PMS was followed by a marked improvement in

behaviour at service: increased libido and better penile rigidity. The quality of semen improved in so far as its density, motility and longevity were concerned, but the percentage of abnormal sperm remained practically unchanged in all cases. An improvement in testicular function was further seen by a decrease in the incidence of testicular cells. The persistence of high abnormalities did not prevent actual conceptions, as the fertility rate increased after treatment. The improvement in semen, especially the sperm numbers, did not last more than a few months following the treatment and had, therefore, to be reinforced by further courses.

The observations show that PMS gives satisfactory results in some cases of degeneration. Even if the sperm abnormalities remain unchanged, an improvement in the density, motility and longevity of semen is enough to raise the fertility rate. Larger and repeated doses appear to give better results. A decrease in the incidence of testicular cells gives a reasonable assurance of the beneficial effects of the therapeutic agent used.

(ii) *Ascorbic acid.*—This was tried in AB 9, 27 and 31. In each case six subcutaneous injections, each containing 2 gm. of the acid in sterile water, were given at four days' interval. AB 9 and 27 showed improvement in sperm density, motility and longevity, followed by a rise in their fertility rates. The number of abnormal sperm and testicular cells, however, increased after the course. It was therefore doubtful whether the semen improvement resulted from improved management and rest or from treatment. AB 31 showed a similar improvement but it was of a very transitory nature, as his semen reverted to its original poor quality in a week's time. This bull had a very high percentage of abnormal heads (10-18 per cent) compared with the other two cases, in which the abnormal heads were within the normal range (2-4 per cent).

In the cases treated, ascorbic acid did not prove satisfactory. Some improvement shown in two out of the three bulls treated was probably the result of improved management and rest. One case which did not show any improvement at all had a very high percentage of abnormal sperm heads. The administration of ascorbic acid in bulls showing a high percentage of abnormal heads therefore seems useless. The increase in the number of testicular cells further gave a reasonable assurance that the theraputic agent employed failed to act.

In view of a very high incidence of testicular degeneration in sterile bulls, it seems essential to explore the possibilities of treating or ameliorating this condition. Some workers are of the opinion that testicular degeneration is incurable, while others hold that it is amenable to treatment, at least in the early stages. The observations made during the course of this investigation concur with the latter view. The chances of recovery are, of course, much greater in the early than in the later stages of testicular degeneration. A promising line of treatment seems to comprise: (1) correcting diet, allowing in particular, proteins in adequate quantity and quality—if necessary, those of

animal origin—together with regular exercise; (2) providing sexual rest whenever necessary; and (3) administering hormones and vitamins. Until more is known of the relation of hormones and vitamins to testicular degeneration, the two should be tried simultaneously. The problem of determining the specific deficiency of a hormone or a vitamin can best be solved by devising simple methods of estimating their levels in urine (androgen content), blood (carotene levels) and, perhaps, semen (male hormone by estimating phosphatase and fructose levels) (Enberg *et al.*, 1947; Mann and Parsons, 1947).

(b) *Testicular hypoplasia.*—The disorder being hereditary and incurable (Klein, 1931; Lagerlof, 1939; Eriksson, 1939), the owners of the affected bulls were recommended to send them for slaughter. The monorchidy of AB 25's brother lent support to the inheritance of this defect.

(c) *Balanitis.*—There was opportunity to treat only one case of balanitis. This was AB 7 with *C. pyogenes* infection. The inflammation being of moderate intensity, " pentaflav " bull cones were employed. The infection yielded to this treatment in three weeks—two cones per week preceded by an antiseptic douche of the preputial cavity each time.

(d) *Paunchiness.*—The condition was corrected by proper dieting and regular exercise. Abnormally large abdomens of AB 2 and 5 became well tucked-up when the daily ration of hay was reduced from 35-40 lb. or more to 17-25 lb., and that of concentrates increased from 3-4 lb. to 8-10 lb., with regular vigorous exercise for about an hour every day.

(e) *Faulty erection.*—PMS was recommended as a trial in two bulls. AB 3 failed to respond to this treatment, while AB 41 is still undergoing the course. If there is no response, it is intended to administer another hormone preparation containing a higher proportion of the lutinising hormone, which stimulates the production of male hormone and is connected with the production of secondary sexual characteristics and erection. Very high amounts of pure lutinising hormone are not likely to be useful owing to its adverse effect on the follicle-stimulating hormone and spermatogenesis.

(2) *Mycotic infection of the prepuce*

While plating preputial washings for bacteriological examination, it was discovered that some of the bulls harboured *Absidia corymbifera* in their prepuce. This was recorded from AB 2, 10 and 12 or in 7.3 per cent of the bulls examined. This was rather a high incidence of mycotic infection. In fact, there was a possibility of discovering more cases if appropriate culture media were employed, as 5 per cent ox blood agar plates used for the purpose are not very suitable for growing moulds. The harmful effects of such an infection could not be traced in AB 10 and 12, but there was sufficient evidence in the case of AB 2 to suggest the venereal transmission of this mould to cows, thereby terminating the pregnancy in abortion (Rollinson and Haq, 1948).

(3) *Incidence of Pseudomonas æruginosa in the prepuce and semen*

Table V shows the incidence of this organism recovered from the preputial washings of sterile bulls.

TABLE V

Incidence of *Pseudomonas æruginosa* in sterile bulls.

Disorder	No. of bulls examined	No. of bulls infected: Total	in semen	in prepuce	in semen and prepuce
Testicular degeneration ...	19	6	—	4	2
Testicular hypoplasia ...	4	2	—	—	2
Balanitis	3	1	—	—	1
Paunchiness	3	1	1	—	—
Faulty erection	3	—	—	—	—
Unknown disorder ...	1	—	—	—	—
Total	33	10	1	4	5
Percentage	100.0	30.3	3.0	12.1	15.2

Table V shows that the infection with *Pseudomonas æruginosa* is quite high among bulls. Although in most cases the organism is found in both the prepuce and semen, in others it occurs only in the prepuce. The infection of semen alone seems to be comparatively rare, and in all probability originates from the preputial infestation. (The preputial hairs were not found to be infected with this organism in the cases reported.) The presence of the organism in all types of bulls does not point to its specificity for any particular disorder.

This organism has been associated with infertility in bulls from time to time (Gilman, 1922; Gunsalus *et al.*, 1944; Henderson, 1946). There is, however, no conclusive proof as to its *modus operandi* in producing infertility in males. The fact that it inhabits the prepuce of apparently fertile bulls shows that the adverse effects of this organism, if any, must take a long time before they become noticeable. In such cases, there is a possibility of a slow and insidious infection resulting in the production of a toxin harmful to sperm. The organism has been seen in the seminal canal as far as the epididymis (with greater concentration in the sigmoid flexure and the seminal vesicles).

(4) *Routine semen collection in testing bulls*

Throughout the investigation, semen was collected by means of an artificial vagina. This method was found convenient and satisfactory in almost all cases. Of a total of 41 bulls tested for infertility, semen could not be collected in five (12 per cent): they were cases of *impotentia cœundi* resulting from faulty erection, paunchiness, etc. Although it was worth while collecting and examining the semen of some of these bulls by ampullar massage, arrangements for the same were not made as the owners did not intend keeping these animals even if their semen was found suitable for use artificially.

A few bulls refused to mount when first brought near the cow, but served later, on walking away from her a few times, employing another cow, changing the place of service or walking the cow in front of the bull and stopping her immediately when he jumped. Some bulls mounted the cow but refused to ejaculate into the artificial vagina. This difficulty was in all cases overcome by making another trial a few minutes or even days later by raising the temperature of the artificial vagina to 45 deg. C. or by allowing the bull to serve once and intercepting the second jump, keeping the artificial vagina away from

his sight all the time and avoiding unnecessary traction on the root of his penis.

Both ordinary cows and those in heat were employed. Of 41 bulls examined, 34 (83 per cent) mounted cows not in heat and 7 (17 per cent) those in heat. Of the latter, 2 bulls mounted cows not in heat on subsequent visits, raising the number of bulls which mounted such cows to 36 (88 per cent). The remaining cases could not be tested further owing to the reasons previously given. Even if it was assumed that these 5 bulls would not have mounted cows not in heat, the number of those which actually did mount them was quite high. Consequently, no difficulty should normally be expected in collecting semen even if a cow in heat is not available. The most important points which came to notice on all occasions were the nature and the size of the cow. Restive cows hampered collection as much as those which could not support the weight of the bull. Such animals could prove dangerous to bulls as well as to the men handling them. The operation was most successful when quiet cows (not heifers) large enough to support the weight of the bulls were used.

E. Summary

The paper records the results of investigations carried out in some parts of Southern England to determine the causes of sterility in bulls. Of 41 bulls tested for infertility, 33 were diagnosed as cases of partial or complete sterility on the basis of clinical examination, semen tests and *post-mortem* findings. Sterility was attributed to testicular degeneration in 19 cases (57.6 per cent), testicular hypoplasia in 4 cases (12.4 per cent), balanitis in 3 cases (9.0 per cent), paunchiness in 3 cases (9.0 per cent), faulty erection in 3 cases (9.0 per cent), and an unknown disorder in 1 case (3.0 per cent). The main results are summarised below.

(a) *Methods of diagnosis.*—(1) A correct diagnosis of sexual maladjustment in bulls is not possible unless a comprehensive examination of the sire and its semen is conducted. Such an examination includes anamnesis, examination of preputial washings, observations of service behaviour, as many semen tests as possible, clinical examination of the internal and external genitalia and a general examination of the sire. The procedure of the examination is described in detail.

(2) A method of recording the service behaviour of a sire, including evidence of its psychogenic reactions, is described and its importance discussed.

(3) A modified method of recording motility in terms of the number of motile sperm and the vigour of sperm movement is described for adoption for bull semen.

(4) The occurrence of degenerating sperm clusters in bull semen is recorded and their diagnostic importance in disease discussed.

(b) *Testicular degeneration.*—(1) In this condition, the fertility rate varied between zero and 50 per cent and did not exceed 25 per cent in 63 per cent of the total number of bulls examined.

(2) Of the total, 66 per cent bulls started their breeding career at the

age of 9-12 months. Putting bulls to service at an early age is suggested as one of the causes of testicular degeneration.

(3) Feeding and management were found to be at fault in most cases. Only 22 per cent of the bulls were fed adequately; the remaining 78 per cent received very little and in some cases too much of concentrates. Arrangements for exercise were unsatisfactory in most cases. The necessity of feeding sires an adequate quantity and quality of proteins from the early age, working out standard rations for bulls under different conditions and making arrangements for regular exercise are pointed out.

(4) Keenness to serve was reduced in 32 per cent bulls, the remaining 68 per cent showing a normal libido. Sexual weakness in 42 per cent of the cases was reflected by variations in penile rigidity, mode of erection, insertion, etc. The importance of observing the latter in every case has been brought out.

(5) A dull or dirty-white colour of semen can be due to the presence of a large number of tissue cells and cytoplasmic debris from the testicles and a yellowish-green colour can denote infection with *P. æruginosa.*

(6) The motility of semen was below normal (70 x 3) in as many as 90 per cent cases and averaged 45.5 x 2.7. The defect lay more with sperm numbers (90 per cent bulls) than with the vigour of sperm movement (42 per cent bulls).

(7) A little over half of the total number of ejaculates, of which some had a poor initial motility and a high percentage of abnormal sperm, showed a longevity of more than 24 hours. As there was no inter-dependence among motility, morphology and longevity, the importance of performing all these tests in each case is stressed.

(8) A fall in sperm density with successive ejaculations (observed in 42 per cent cases) and counts lower than 600,000 sperm per c.mm. (observed in 37 per cent cases) are shown to suggest a derangement of testicular function.

(9) The permissible limit of the abnormalities of the head, middle-piece, tail and tailless heads or headless tails are fixed at 4, 8, 2, and 6 per cent (total 20 per cent) for normal semen. The average figure for the percentage of abnormal sperm in the fertile bulls examined was 15.1 per cent (min. 6, max. 26).

(10) The number of abnormal sperm in testicular degeneration averaged 31.4 per cent (min. 15, max. 100). Of the total, 21 per cent bulls showed very little or no increase in abnormal sperm, whilst in 79 per cent cases the abnormalities of the head (32 per cent), middle-piece (16 per cent), tail (5 per cent), headless tails or tailless heads (5 per cent) and mixed abnormalities (21 per cent) were readily detectable. The abnormalities of different types varied from normal limits to as many as 18 per cent abnormal heads, 38 per cent abnormal middle-pieces, 26 per cent abnormal tails, and 91 per cent tailless heads or headless tails.

(11) A marked rise in the percentage of abnormal heads, particularly those of the eosinophilic variety, together with a poor sperm density, is suggested as an indication of defective spermatocytogenesis. An increase in the per-

centage of abnormal middle-pieces, when other parts of the sperm are normal and the sperm density is within the normal range, is suggested as an indication of defective spermiogenesis. Abnormalities of mixed type are taken to indicate defective spermatogenesis. The importance of the abnormalities of the middle-piece and tail is stressed.

(12) Changes in the size and consistency of the testicles were detectable in 79 per cent bulls. Of these, 26 per cent had testicles of normal size but soft and flabby consistency, and 53 per cent of subnormal size with or without a change in their consistency (turgid 26 per cent, soft and flabby 21 per cent, and normal 6 per cent). A soft and flabby consistency was generally accompanied by an increase in the percentage of abnormal sperm.

(13) A tucked-up position of the testicles, an abnormally long spermatic cord and an abnormal fat deposition in the scrotum are discussed in relation to infertility.

(14) A high incidence of the spermatogenic cells having pyknotic nuclei in the testicular tubules of a bull is held responsible for 18-21 per cent sperm with under-sized heads present in his semen during life.

(15) The diameter of the seminiferous tubules was not reduced markedly in testicular degeneration. In the four cases studied it averaged 202 μ (min. 116, max. 320).

(c) *Testicular hypoplasia.*—(1) Owing to a very lively service behaviour of the bulls in this group, the owners did not suspect sterility and incurred loss by keeping the animals for as long as six months to a year.

(2) Of the four cases, one had a fertility rate of 10-40 per cent, and the other three were completely sterile.

(3) In two bulls the semen showed a deposit of ammonium urate.

(4) Semen was very poor in motility and averaged 6.8 x 0.7 (min. 0, max. 20 x 2), and the sperm did not live for more than 18 hours in any case.

(5) The density decreased with successive ejaculations, showing suppressed spermatogenesis and poor reserves in all cases. The number of sperm averaged 42,700 (min. 0, max. 121,000) per c.mm. The milky colour of an ejaculate does not necessarily indicate the presence of sperm.

(6) The percentage of abnormal sperm averaged 39.1 (min. 22, max. 58), figures comprising abnormalities of different types. An increased incidence of abnormal heads up to 15 per cent was observed.

(7) An under-development of the epididymis in young infertile bulls is suggested to be taken as an aid in diagnosing testicular hypoplasia during life. In one case, one-sided vacuolar degeneration of the tail of the epididymis was recorded.

(d) *Balanitis.*—(1) Balanitis was responsible for *impotentia generandi* in one bull and *impotentia cœundi* in another. One bull carrying *T. fœtus* did not show the commonly reported sluggishness at service.

(2) An assessment of the depth of an injury to the preputial mucosa on cytological evidence is suggested as an aid in diagnosing *T. fœtus* infection when the actual parasites cannot be demonstrated.

(e) *Paunchiness.*—(1) The adverse consequence of feeding bulls on a very large quantity of hay is brought out and the importance of the condition in rendering bulls useless for breeding purposes discussed.

(2) The development of the vice of masturbation due to a long period of idleness, consequent upon a large pendulous abdomen and inability to mount the cow, is described.

(f) *Faulty erection.*—Three cases of faulty erection, in which the penis did not come out of the prepuce for more than 3-6 inches, are described. All of them showed a stiffness of the lumbar region and a peculiar lameness of the right hind leg. The causes of the condition through injuries to the lumbo-sacral and perineal regions are discussed.

(g) *Additional notes.*—(1) Pregnant mare's serum was tried in three cases of testicular degeneration. After treatment, the service behaviour of bulls improved and their semen showed higher density and better motility and longevity, but the percentage of abnormal sperm remained practically unchanged. The fertility rate rose in all cases. Better results were obtained with larger and repeated doses.

(2) Ascorbic acid tried in three cases of testicular degeneration failed to effect improvement in semen or fertility rate. Its use appears futile when large numbers of abnormal heads were present in semen.

(3) Variations in the incidence of tissue cells (other than spermatozoa) in the semen of the treated bulls served as a useful guide in determining the effects of the therapeutic agents on the reproductive organs.

(4) In view of a very high incidence of testicular degeneration, the necessity of treating or ameliorating this condition in bulls is emphasised.

(5) The occurrence of *Absidia corymbifera* in the prepuce of 7.3 per cent bulls is recorded and its importance in the causation of sterility described.

(6) Of the sterile bulls examined, 30.3 per cent were infected with *Pseudomonas æruginosa.* It was recovered from both the prepuce and semen in 15.2 per cent, from the prepuce alone in 12.1 per cent, and from the semen alone in 3.0 per cent cases.

(7) Of the total, 83 per cent bulls mounted cows not in heat, 5 per cent those in heat, and 12 per cent did not mount, either due to *impotentia cœundi* or because the cow was not in heat. A few notes on semen collection, when bulls refuse to mount or ejaculate into the artificial vagina, are given.

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(To be continued)

BLEEDING HOGS

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The description by Carle and Dewhirst[1] of a method of bleeding swine from the anterior vena cava has greatly facilitated the fight against Brucellosis of swine. Prior to this time, blood samples were obtained from the tail or the ear veins. Both of these methods had limitations. The method of Carle and Dewhirst is safe, rapid and easy to perform.

The anterior vena cava lies in the thoracic inlet between the first pair of ribs and gives rise to the external jugulars and the right and left brachial veins. In order to puncture the anterior vena cava the needle is inserted on an imaginary line from the base of the ear to the point of the sternum. In the standing animal the needle enters the neck 1 to 3 inches from the point of the sternum, depending on the size of the animal, and is directed upward, inward and backward, aiming at the mid-point between the first pair of ribs. When the vein is entered in this area the blood flows rapidly and enough for a Brucellosis test (6 c.c.) can be obtained in a few seconds.

One thing that has held back wider adoption of this method has been the lack of proper equipment. Few veterinarians have the three to four-and-a-half inch needles necessary for bleeding large hogs. We have been unable to get blood in large thick-necked hogs with a three-inch needle, but have always been successful with a four-and-a-half inch needle. Other equipment and the procedure used in obtaining the blood sample is as follows:

1. The hog is trapped in a chute or with a hurdle in a corner of the fence. A lariat noose with quick release or a hog holder is placed behind the tusks and the hog pulled up to a post, with the snout elevated as much as possible.
2. The neck is washed with a water-soaked cloth and, if necessary, followed by cotton-wool soaked in antiseptic solution.
3. A 3-inch 19 gauge needle or a 4½-inch 18 gauge needle and a 10 c.c. glass syringe are used to draw the blood.
4. The needle is removed from the syringe (to prevent hæmolysis) and the blood discharged slowly down the side of the blood vial.
5. The needle and syringe are then rinsed in cold, clear water and the needle placed in a pan of antiseptic solution.
6. A second needle is placed on the syringe and rinsed in sterile sodium citrate solution.
7. The necessary records are made and we are ready for the next hog.

Following this procedure one can, with a little practice, bleed hogs as rapidly as they can be caught. At a recent meeting this method was described and 14 practitioners who had never used it were able to get blood on the first attempt.

We have bled a few mature hogs with a 3-inch 16 gauge California type

(1) Carle, B. N., and Dewhirst, Wm. H., Junr.: "A Method for Bleeding Swine," *J.A.V.M.A.*, 101: 495-496, December, 1942.

bleeding needle. This worked very satisfactorily and eliminated the necessity for the glass syringe. It is not long enough for old, large hogs. We have hesitated to try this large-bore needle on any but mature animals.

A few difficulties were encountered while learning this method. It was found that with older hogs the needle needed to be inserted further up the neck from the sternum. This is necessary to locate the middle of the thoracic inlet and to avoid bone deposits in the region of the sternum. If bone is hit the needle should be withdrawn and the plug of periosteum that usually enters it removed. The needle should then be redirected, for bone is not encountered when the needle is correctly inserted. When starting the needle through the tough skin, a short, sharp jab directly in line with the axis of the needle will prevent it from bending and consequently being misdirected. When the needle reaches the vicinity of the anterior vena cava, a jabbing motion will facilitate the penetration of the vein. Slight backward pressure on the barrel of the syringe aids in locating the vein immediately after it is punctured. If this is done a satisfactory sample is often obtained from the external jugular after penetrating about 1½ inches. Elevation of the snout makes the area of operation more easily accessible.

Restraint of the hogs is easily accomplished with a hog holder or lariat wrapped around a post. We prefer the latter with a quick release as it is easier on the help. The hog holder has the advantage of being easier to manipulate. Catching the hogs offers varying difficulties, depending on the facilities available on different farms. They can be caught readily in a chute after being driven to the end and prevented from going backward. Almost every stock farm will have a stout corner in the stock yard where the animals can be hemmed in with a portable panel or hurdle and snubbed up to a post.

After the samples are collected they should be sent directly to the laboratory with the request to run them at the 1-25, 1-50 and 1-100 dilutions. If they must be kept any length of time, they should be stored in the refrigerator. Samples that will be in transit a long time should be allowed to clot and the serum poured into another tube for shipment.

Medication can also be given by this route. Many practitioners use this method for intravenous anæsthesia with pento-barbitol sodium for such operations as reduction and repair of hernia.

CHEMICAL INVESTIGATION OF THE EGYPTIAN PLANT, EUPHORBIA PEPLUS

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Introduction

FROM the earliest times, the ingestion of *Euphorbia peplus,* Lin., has been noted to cause toxic symptoms of painful vomiting and purgation in domesticated animals. All that can be traced in the literature relates to species of Euphorbia other than *Euphorbia peplus,* which is the common Egyptian species. Even in

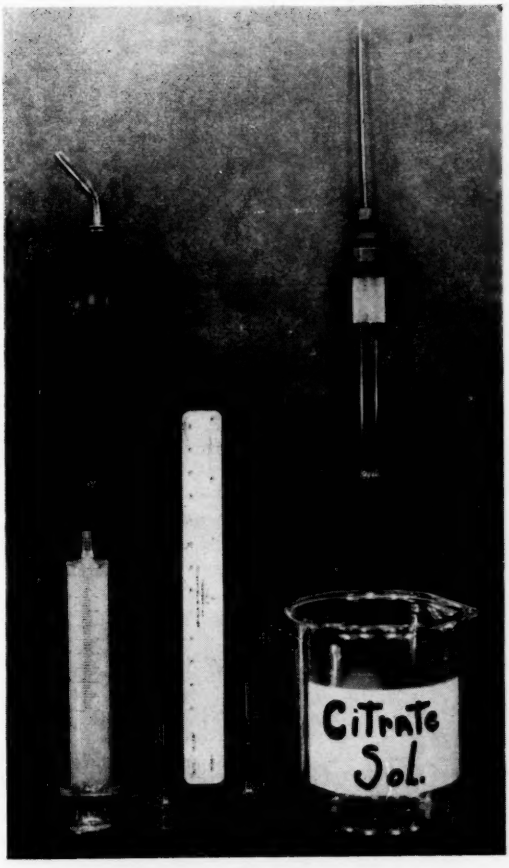

FIG. 1

Instruments required. Ruler is 6 in. (inclus.) long.

(*Article by Sippel on page 127.*)

FIG. 2

Bleeding from Vena Cava standing position. Restraint by snare over upper jaw.

FIG. 3

Bleeding in standing position. Restraint by snare over upper jaw.

(*Article by Sippel on page* 127.)

regard to those other species, no full investigation into their active principles has been carried out. All that can be found in the literature amounts to a suggestion by some authors of the presence of an alkaloid. Other authors, however, have believed that the activity of the plant is mainly due to a resin.

The Euphorbiaceæ form one of the most important botanical orders, containing useful medicinal plants and the Euphorbia forms the most important genus within the order. Although *Euphorbia peplus* has been stated to be of importance in medicine, little is known about its medicinal properties. The plant is exceedingly prevalent in fields of barseem (= Egyptian clover), which is the main fodder of domesticated animals in Egypt.

Part I. History, Etymology, Description and Distribution

History.—Euphorbia is the old Greek name given to an African plant characterised by its extreme acridity, and was so named after Eupherbus, physician to King Juba II of Mauritanea A.D.18, who was the author of several books including treatises on Euphorbia. Fluckiger says that the plant is mentioned by numerous other early writers on medicine such as Galen in the second century, Vindicianus in the fourth century, Otius in the sixth century, and Paulus Ægeneta in the seventh century A.D., and it was also known to the Arabian School of Medicine. Hence, the drug had a place in all the early Pharmacopœas. Long afterwards, some of its species were examined and differentiated. For example, the plant yielding the resin euphorbium was first described at the beginning of the 18th century by an English merchant named Jackson who had resided many years in Morocco. From the figures he published the species cannot be accurately identified. In 1849 it was mentioned in the "Admiralty Manual of Scientific Enquiry" that the plant yielding euphorbium is *Euphorbia resinefera.* Martindale states that *Euphorbia pilulefera* was first introduced by Can-Boyd, of Townsville, Queensland, in 1880 as an asthma remedy and became known as Queensland asthma herb. In 1934, I. R. Fahmy and H. Kamil gave a full description of *Euphorbia prunifolia,* one of the most prevalent Egyptian species of Euphorbia. Afterwards other species have been described. In 1940 the present writer began an exhaustive study of *Euphorbia peplus;* the chemical part of which is here summarised.

Etymology.—The plant is known specifically as *Euphorbia peplus,* taking its specific name from the Latin word denoting the small size of the species. In the English literature it is known by different names depending on the action attributed to it: petty spurge (small purge), wart weed (for painting warts), devil's milk and diels milk (its secretion having a milky appearance and acting harmfully), etc. In Egypt it is known also by several names according to its action: *libbeynah* (excreting a milk-like substance), *sabun-gheyt* (used by the fellah in washing his hands), *harrarah* (due to its vesicant action), *kabdah* (for its toxic and fatal effects), and *laban el sodah* (due to its harmful effects). It is also known by other names according to the locality.

Description.—*Euphorbia peplus* (Fig. 1) is an annual plant, small, glabrous,

light green, 20-30 cm. high, branching near the apex, erect. Leaves are tender, entire, petioled, obovate 0.5-2.5 cm. long, obtuse or retuse. Umbel of repeatedly forked rays often occupying the greater part of the plant. Capsule is ovate, 1.5

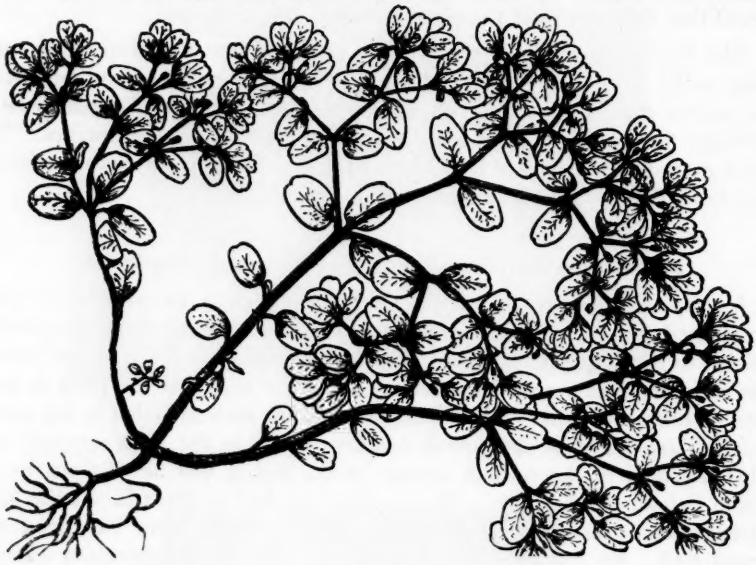

FIG. 1
The flowering *Euphorbia peplus* plant (two-thirds natural size).

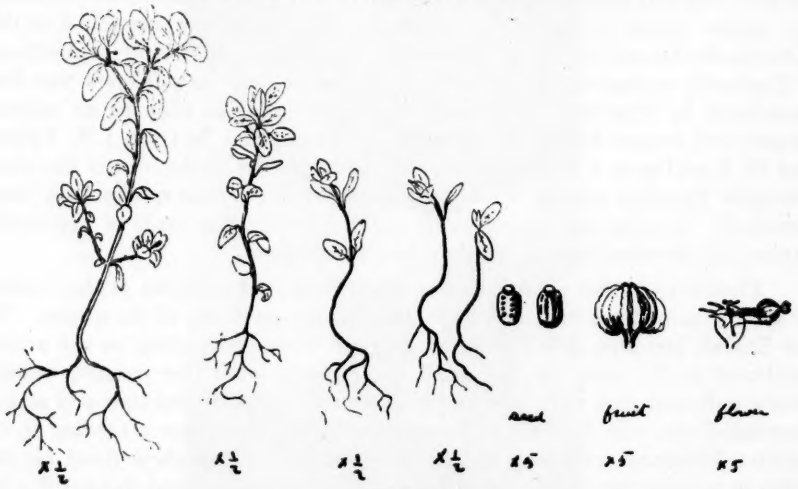

FIG. 2
Different stages of growth of plant.

mm. long. Seeds (Fig. 2) small, ovate, oblong, with a longitudinal groove on each of the two compressed ventral sides, and on the dorsal side there are two rows of 4 pits each, light grey in colour with a whitish caruncle attached to the

upper end of each seed. According to personal observations, the plant was found to flower usually at the end of November and sometimes a few weeks later, especially in cold weather or on damp soil.

Distribution.—The location of this plant in Egypt is along the Mediterranean coast, in the Nile Delta region, and in Fayoum and some of the oases. It can also be found to some extent in all parts of the country, even as far south as Aswan. It grows usually in cultivated lands amongst barseem and other crops, on the banks of canals and in gardens, where it grows in patches alongside hedges and in the neighbourhood of trees as if seeking protection from extreme weather conditions.

Part II. Analysis of the Plant and Isolation of its Active Principles

A. *Preliminary Chemical Investigation.*

1. The plant was collected, cleaned, dried, finely powdered, sieved, and the powder then subjected to preliminary tests to obtain an indication of its active constituents.

2. A few grams of the powder were extracted with boiling alcohol (95 per cent) and filtered. The filtrate was diluted with distilled water slightly acidulated HCl. A heavy resinous precipitate was formed, indicating the presence of resins in the plant.

3. Another few grams of the fine powder were extracted with warm water containing about 1 per cent HCl and then filtered. On testing the filtrate with the usual alkaloidal reagents (Mayer's reagent, Wagner's reagent, Chagre's reagent, etc.) a precipitate was given by all, immediately indicating the probable presence of alkaloids.

4. An aqueous extract of the powder, made with boiling water, reduced Fehling solution, giving on boiling a faint greenish precipitate. After hydrolysis with HCl, and rendering the solution alkaline, a red precipitate was however given with Fehling solution, indicating the presence of reducing substance after hydrolysis.

5. The aqueous extract made with boiling water also gave with ferric chloride a deep green coloration, indicating the presence of tannin. It gave with iodine solution a very faint bluish colour.

The above preliminary tests indicated the presence of a resin, alkaloids and glucosides.

B. *Separation of the Active Principles.*

The Resin

1. *Isolation from the powdered plant.*—The fine powder, 10 g. by weight, was placed in a flask with 100 c.c. of 95 per cent alcohol. The flask was tightly corked and left in an incubator at about 50 deg. C. for about 20 hours, shaking it from time to time. The filtered liquid extract was placed in a suitable beaker and evaporated on a water bath down to 25 c.c. Distilled water, slightly acidulated, 50 c.c., was then added and the mixture gently warmed until all the alcohol was driven off. On cooling, the resin settled and then deposited

completely. The deposited resin was repeatedly washed, then dried in a hot-air oven at 105 deg. C. and weighed. This was found to range between 7 and 8 per cent of the dry powder as shown by several extractions.

2. *Isolation from the alcoholic extract.*—The resin was also separated from the alcoholic extract, prepared by moistening the powdered drug with ammonia and alcohol, packing in a continuous extraction apparatus, and then extracting with 95 per cent alcohol until completely exhausted, the alcohol was completely evaporated and the residue, dried in an air-oven at 105 deg. C., was found to weigh about 24 per cent of the dried plant. By taking a known weight of this dried extract, dissolving in the least possible amount of alcohol, precipitating the resin by adding a large volume of water slightly acidulated with HCl, filtering the precipitated resin and washing it with water until freed from impurities, the resin obtained after drying was found to weigh about 31 per cent of the extract.

The Alkaloid

1. *Isolation of the alkaloid from the alcoholic-extract.*

(a) The dried alcoholic extract was treated with several portions of acidulated water, which were each time filtered off until the alcoholic extract was completely exhausted.

(b) The acid filtrates were mixed in a separating funnel and washed with chloroform to remove impurities, especially resins. The chloroform was then rejected.

(c) The acidic solution was rendered alkaline with ammonia and extracted with successive portions of chloroform until completely exhausted.

(d) The chloroform extracts were mixed and then repeatedly extracted with 1 per cent HCl until completely exhausted.

(e) The acidic portions were mixed, rendered alkaline with ammonia, and then again shaken with successive portions of chloroform until completely exhausted.

(f) These chloroform extracts were mixed and washed twice with a few c.c. of distilled water, and the water was rejected. Anhydrous sodium sulphate was added to the chloroform to remove water and clarify the solution, which was then filtered.

(g) The chloroform was evaporated and the residue dried and weighed. It was found that the alkaloidal residue amounted to about 0.5 per cent of the dried alcoholic extract.

(h) Alkaloidal salts, sulphates, hydrochlorides and tartrates were prepared by neutralising the alkaloidal residue with the respective acids, filtering and leaving the filtrate to evaporate, when crystals of the salts were formed.

2. *Isolation of the alkaloid from the powdered plant.*

(a) The finely powdered plant was extracted by macerating with sulphuric acid 1 per cent for four days, shaking from time to time.

(b) The liquid was then filtered away, the solid mass pressed and extracted once more with another portion of 1 per cent sulphuric acid for four days until completely exhausted and filtered.

(c) The resulting mixed filtrates were clarified by repeated filtration. The clear filtrate so obtained was transferred into a separating funnel and made alkaline with ammonia.

(d) The turbid alkaline fluid was extracted with successive quantities of chloroform to dissolve the precipitated alkaloid and the chloroform extract then shaken with successive portion of 1 per cent HCl. The mixed acidic washings were again rendered alkaline with ammonia and extracted as before with several portions of chloroform.

(e) The mixed chloroform extracts were evaporated to dryness. The alkaloidal residue was found to weigh about 0.13 per cent of the powdered plant.

The Glucoside

1. *Isolation of the glucoside from the powdered plant.*

(a) The powder, 100 g., was extracted until exhausted with successive portions of boiling distilled water to which calcium carbonate had been added.

(b) The aqueous extracts were mixed and treated with lead acetate solution until no more precipitation took place. The lead precipitate was filtered and the filtrate again treated repeatedly with strong lead subacetate solution until no more precipitation took place. The excess of lead in the mixed filtrate was removed by passing H_2S gas, and excess of this was driven off by passing a current of air.

(c) The clear filtrate was then evaporated under reduced pressure at low temperature nearly to dryness, and the residue dissolved in the least possible amount of boiling absolute alcohol, filtered while hot, and then left in an ice-chest to cool.

(d) On evaporating this alcoholic solution slowly in a dessicator a solid residue was obtained. This crude residue was purified by redissolving it in absolute alcohol, cooling and gradually adding ether to it until turbidity occurred. It was then kept in an ice-chest, when a crop of sugary substances separated out. The supernatant liquid was decanted into another container and more ether added to it until a crystalline deposit was obtained. The addition of ether to the absolute alcohol solution was continued until no more crystals separated out.

(e) The crops of crystals were collected, weighed, and found to amount to about 3 per cent of the powdered plant.

2. *Separation of the glucoside from the alcoholic extract.*

(a) The alcoholic extract of the powdered plant was taken up in boiling distilled water, and the aqueous solution then filtered.

(b) The procedure as described above, from b onwards, was then repeated.

Discussion and Conclusions

1. In regard to its botanical characters, the Egyptian plant, *Euphorbia peplus,* was found to agree with the descriptions of the plant given by other writers in other countries, with the following exceptions:

(a) All leaves are exstipulate, and no stipules were found in the floral axis as is stated in the British Pharmaceutical Codex.

(b) The pits of the dorsal sides of the seed were always found to be arranged

in two rows of four pits each, which arrangement has not been described hitherto.

(c) The flowering period of the plant was found to start at the end of November or a few weeks later, instead of March, as was found by R. Muschler and others.

2. In regards its chemical investigation, it was found from the above approximate analysis of the air-dried *Euphorbia peplus* grown in Egypt that it contains about 5.5 per cent of resin, 0.13 per cent of alkaloidal residue, and about 3.0 per cent of glucosidal substance. Thus it agrees to a certain extent in its active constituents with *Euphorbia pilulifera* which contains, according to Kraemer, an alkaloid 0.1 per cent, a glucosidal substance 0.4 per cent, and several resins. Gaston Bonnier is the only authority who has mentioned something about the constituent of *Euphorbia peplus*. He states that it contains 4.8 per cent resin, 9.75 per cent gum, 2.15 per cent salt, and 88 per cent water, but he does not mention the presence of any glucosidal or alkaloidal principles to whose presence, together with the resin, the Egyptian plant mainly owes its activity and toxicity.

3. The isolated active constituents possess the following characteristics. The precipitated resin is sticky, semi-solid in consistency, of a dark brownish-green coloration. The alkaloid occurs as an amorphous or, rarely, as microcrystalline powder. It turns red litmus paper blue. Its solution in dilute acids gave with the alkaloidal reagents immediate precipitates. It is insoluble in water, but soluble in organic solvents. It is positive to sodium fusion tests for nitrogen. The sulphate of this alkaloid exists as needle-shaped crystals; the hydrochloride as short needle-shaped or branched crystals, and the tartrate in the form of leaf-like crystals. The isolated glucoside was found to occur as long needle-shaped colourless crystals, possessing a very slight bitter and acrid taste, readily soluble in cold distilled water and in hot alcohol, and insoluble in neither ether nor chloroform. It does not reduce Fehling's solution except after hydrolysis, indicating its glucosidal nature.

4. The activity of these principles was tested, thus, the resin was found to be acrid, irritant to the mucous membranes, and was experimentally found to produce purgation and vomiting in dogs, while the alkaloid and the glucoside were found to produce dilatation of the blood vessels, lowering of the blood pressure and other actions indicating their activity.

Acknowledgment

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REVIEWS

Artificial Insemination in North and Western Europe (Notes and Observations from a visit to Denmark, France, Great Britain, Holland and Sweden). By T. Bonadonna. Collana Technico-Scientifica, "L. Spallanzani," N.6.

During 1948, Professor Bonadonna, who is the well-known Director of the Instituto Sperimentale Italiano "L. Spallanzani" in Milan, visited Sweden, Denmark, Holland, France and England for the purpose of collecting information about the present status of artificial insemination techniques and organisation in these countries. He gives presumptive estimates of the number of cows submitted for artificial breeding in the years indicated as follows: Sweden (1946), 4,600, and (1948), 110,000; Denmark (1947), over half a million, i.e. 35 per cent of the total national dairy herd; France (1948) and England (1948), 300,000; Holland (1947), despite impeding restrictions, 44,000.

Professor Bonadonna indicates that in the countries he visited the organisation of A.I. centres was generally based in breeders' associations or co-operative societies, which operated without profit motive for the purpose of improving their members' cattle by the use of superior bulls.

In some of the countries of his tour, Bonadonna found that veterinarians were not available for A.I. work and that the mere routine procedure of semen injection was carried out by trained technicians (i.e. lay inseminators). Because of the possibility of subsequent "professional competition" on the part of these technicians, Lagerlöf in Sweden was trying to circumvent the latter eventuality by utilising women inseminators, who had first undergone a training period of 4-5 months at the Veterinary College of Stockholm.

Legislation on A.I. varied in the different countries; in France, where organised artificial breeding was relatively young, it is said that the authorities, profiting from the previous experience of other countries, had instituted enactments on the subject which were both extensive and detailed. Holland was the only country where the practice of A.I. is forbidden, save under definite conditions of specific derogation.

As regards A.I. in Britain, Professor Bonadonna seems to have obtained a correct overall picture of the set-up, and also of the relation of this method of breeding to livestock improvement as we, in this country, see it.

"OUR PLUNDERED PLANET," by Fairfield Osborn. London: Faber & Faber, Ltd. Price: 10s. 6d.

THIS book is yet another statement of the now fully recognised position of mankind in relation to agriculture. Will he survive or will he continue to multiply so fast and to destroy the soil so rapidly that he will exterminate himself? It is touch and go. Lord Bryce in the House of Lords recently described the situation as "precarious beyond words." Lord Boyd Orr thinks there is a chance that man will come to his senses before it is too late, but with all the forces of economic pressure playing their part; with the almost complete ignorance of the general public of what is going on in the world; with the impelling desire of governments to cover up the truth; with all these things and many more fighting against the proper conservation of the soil, the chances of mankind surviving on this earth for long certainly look slim.

Mr. Fairfield Osborn's exposition is crystal clear and is easily read and easily understood. The publishers quote Albert Einstein as saying of his book that "reading it one feels very keenly how futile most of our political quarrels are compared with the basic realities of life," which might be said to be an almost trite observation, but it would not be trite to say that the tragedy of this modern age is that it is being wilfully blind to its own very existence. We commend this book to all veterinary surgeons. No man who expends the price of three packets of cigarettes upon it will ever have expended money to a better purpose. Why so? Because, in the words of Mr. Fairfield Osborn, "the problem is how to conserve the remaining good natural soils that exist on the earth, together with the complementary resources of forests, water sources and the myriads of beneficial forms of animal life. There is no other problem. If that is not solved the threat to human life will grow in intensity and the present conditions of starvation that are already apparent in various parts of the earth will seem as nothing in the years that lie ahead."

Another valuable new book on this subject has lately been published called, quite appropriately, "The Road to Survival," by William Vogt, chief of the Conservation Section of the Pan American Union, but the publishers seemingly do not think veterinary surgeons likely to be interested in this small matter of survival and so did not send us a review copy. Nevertheless, we commend this book also to our readers' attention. It is as interesting as it is alarming.

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